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Development of an *E. coli* 0157:H7 Specific Probe

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E. coli strain 0157:H7 is an organism causing severe bloody diarrhea in humans. It's often contracted by ingestion of contaminated food. Present methods used for this diagnosis are time consuming, expensive and not of great sensitivity. The attempt to develop the DNA probe was to circumvent problems of present methods and provide better health care. The 60 megadalton plasmid of *E. coli* strain 0157:H7 was isolated by a modification of the Magic Miniprep method [Promega Scientific, Madison WI]. The plasmid was hydrolyzed with the restriction endonuclease Pstl, ligated into pUCi8; the recombinant molecule was used to transform competent DH5 *E. coli* cells. Approximately 35 clones were obtained from the 0157:H7 plasmid library. This report ends the work on ILIRAE31. Further testing will be conducted under North East Research Cancer Institute Grant JON 725011XX.

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TECHNICAL NOTE

Development of an Escherichia *(E. coli)* 0157:H7-Specific Probe

E. coli strain 0157:H7 is an organism causing severe bloody diarrhea in humans. It's often contracted by ingestion of contaminated food. Present methods used for this diagnosis are time consuming, expensive and not of great sensitivity. The attempt to develop the DNA probe was to circumvent problems of present methods and provide better health care.

The 60 megadalton plasmid of *E. coli* strain 0157:H7 was isolated by a modification of the Magic Miniprep method [Promega Scientific, Madison WI]. The plasmid was hydrolyzed with the restriction endonuclease Pstl, ligated into pUCi8; the recombinant molecule was used to transform competent DH5 *E. coli* cells. Approximately 35 clones were obtained from the 0157:H7 plasmid library.

The resulting recombinant DNA molecules were isolated from the clones; these were hydrolyzed with Pstl to free the 0157:H7 inserts. The Pstl-cut recombinant DNA molecules were then electrophoresed and Southern-blotted onto nitrocellulose paper. The blots were probed with digoxygenin-labeled DNA of various plasmids found in other strains of *E.coli* -such as strains 88, 189, 221 that are responsible for various enterpathogenic disorders in humans. Insert DNA bands which did not hybridize with these plasmids were assumed to be possible 0157:H7-specific probe candidates. Eight clones contained such inserts. The inserts ranged in size from 1500 to 350bp. Sequencing of each insert is now in progress so that a ploymerase chain reaction [PCR] system can be developed. Once sequencing is completed, clinical trial will begin.

This report ends the work on ILIRAE31. Further testing will be conducted under North East Research Cancer Institute Grant JON 725011XX. In clinical trials conducted by the Clinical Bacteriology section specimens will be submitted to the Molecular Biology Institute at the University of Scranton, Scranton, PA.